Original Report

Transmission Patterns of Tuberculosis in Taiwan: Analysis by Restriction Fragment Length Polymorphism

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ABSTRACT

Objectives: To study the pattern of tuberculosis transmission in Taiwan.

Methods: An insertion sequence IS*6110*-based restriction fragment length polymorphism (RFLP) analysis of *Mycobacterium tuberculosis* isolates was conducted.

Results: Among 85 patients with culture-confirmed tuberculosis diagnosed between August 1993 and March 1994, 79 (93%) had isolates with unique RFLP patterns, whereas 6 (7%) had isolates that belonged to three clusters of identical RFLP patterns. Two strains in one cluster were isolated from patients who lived in the same small village. The average age of the patients was 54 years, and more than 40% had an underlying medical problem. The high degree of diversity of the RFLP patterns, and the demographic and clinical characteristics of the patients suggest that, in Taiwan, a large proportion of newly diagnosed tuberculosis cases are attributable to reactivation of an infection acquired in the remote past. These observations contrast with those in communities with a high prevalence of human immunodeficiency virus (HIV) infections, where a large proportion of new tuberculosis cases may be attributable to recent infections.

Conclusions: The findings suggest that the distribution of *M. tuberculosis* with identical RFLP patterns (representing recent transmissions) may be greatly influenced by the prevalence of HIV infections in the community. In addition, the steady decline in the incidence of tuberculosis in Taiwan, despite its relatively high incidence, suggests that the trend in disease incidence may be influenced by the prevalence of cluster strains in the community.

Key words: *Mycobacterium tuberculosis, restriction fragment length polymorphism, Taiwan, tuberculosis*

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Although the incidence of bacteriologically proven tuberculosis has been steadily declining in Taiwan since 1957, it is still substantially higher than in the United States.[1,2] In 1993, the incidence of pulmonary tuberculosis in Taiwan was approximately 50 cases per 100,000 population, whereas in the United States it was 9.8 cases per 100,000 population. It is not known what proportion of the new cases of tuberculosis in Taiwan are attributable to recent infection or reactivation from an old infection. The use of insertion sequence IS*6110* to generate restriction fragment length polymorphism (RFLP) patterns of *Mycobacterium tuberculosis* has been widely used to subtype clinical isolates and characterize transmission patterns of tuberculosis.[3–13] Recent studies from San Francisco and New York City suggested that approximately 40% of new tuberculosis cases may be attributable to recent exogenous infections.[14–16] Such a pattern of transmission may be influenced by the prevalence of human immunodeficiency virus (HIV) infections in these communities. In a 10-year period between 1984 and 1993, only 93 cases of acquired immunodeficiency syndrome (AIDS) were reported from all of Taiwan, half of which were reported in the last 2 years. The authors conducted a study of IS*6110*-based RFLP analysis of *M. tuberculosis* in an urban hospital in Taiwan with a low prevalence of HIV infections, to examine the pattern of transmission of tuberculosis.

MATERIAL AND METHODS

Mycobacterial Strains

All culture-proven tuberculosis cases consecutively diagnosed at Mackay Memorial Hospital in Taipei, Taiwan, from August 1993 to March 1994, were included in this

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study. The isolates were subcultured onto Lowenstein-Jensen (Difco, Detroit, MI) slant and transported to the United States with the permission of the Centers for Disease Control and Prevention (Permit No. 94-266-2). Mackay Hospital is a referral center for Taiwan island that also provides primary care. Approximately 350 patients with active tuberculosis are admitted per year to this hospital. Most of the patients come from greater Taipei County and Taipei City.

Demographic, Clinical, and Epidemiologic Features

Medical records of all patients with mycobacterial isolates were reviewed: residence, sex, age, date of hospitalization, underlying medical conditions, source of clinical specimens, and date of isolation were recorded. If isolates with identical RFLP pattern were cultured on the same day, they were interpreted to represent laboratory contamination, and hence excluded from analyses. Evidence of close contact or other potential opportunities for transmission, such as medical procedures, visits to the same clinic on the same day, or sharing the same inpatient hospital room, were reviewed. The clinical data were obtained by a physician who was blinded to the RFLP results.

Procedures and Criteria Defining a Cluster

Restriction fragment length polymorphism analysis was performed according to a standardized procedure described by van Embden et al.[13] Briefly, the mycobacterial DNA was extracted, digested with PvuII, resolved by agar gel electrophoresis, and transferred by the method of Southern to a nylon membrane. The target DNA, representing the repetitive DNA element IS*6110*, was hybridized with a probe generated by polymerase chain reaction (PCR)-amplified sequences within IS*6110*. In each gel, DNA was included from an H37Rv strain of *M. tuberculosis* and from a clinical isolate that yielded regularly spaced bands as reference strains. Supercoiled DNA ladder and λDNA/Hind III fragments (Life Technologies, Gaithersburg, MD) were also included in each gel as molecular weight markers. The IS*6110* bands in the membrane were visualized by a nonradioactive chemiluminescent method according to manufacturer's instructions (Boehringer Mannheim Biochemicals, IN). The RFLP patterns of the different isolates were compared visually. If there was any doubt of similarity of patterns, the procedure was repeated and samples were run on the same gel.

Isolates with RFLP patterns with identical number and molecular size position of the bands were considered to be related, and were defined as having clustered RFLP patterns, whereas isolates with unique RFLP patterns were deemed unrelated and hence nonclustered. As in previously reported studies,[14-16] strains with clustered patterns were assumed to be isolates from patients with tuberculosis who acquired the infection recently (primary tuberculosis), whereas strains with unique patterns were assumed to be isolated from persons with reactivation tuberculosis.

RESULTS

Clinical Characteristics

Between August 1993 and March 1994, 90 patients with culture-proven tuberculosis were identified. Five of them were excluded from analysis because of contamination or nonviable cultures. The isolates of the remaining 85 patients were available for RFLP analysis. The patients represented about 25% of the annual number of new tuberculosis cases diagnosed at Mackay Hospital.

The age of patients ranged from 7 to 86 years (mean, 54 y). The male:female ratio was 2.9:1. The sources of specimens included sputum (n = 78), wound drainage (n = 4), cerebrospinal fluid (n = 2), and gastric aspirate (n = 1). Clinical manifestations included pulmonary tuberculosis (n = 78), tuberculous meningitis (n = 2), cutaneous tuberculosis (n = 2), and one case each of miliary tuberculosis, spinal tuberculosis, and tuberculous pleurisy with pleurocutaneous fistula. More than 40% of the patients had an underlying medical problem (Table 1).

Analysis

All mycobacterial strains available for RFLP analysis were initially shown to be positive by the PCR test for IS*6110* sequences. The *M. tuberculosis* "fingerprinting" showed that isolates from 79 (93%) patients had unique RFLP patterns (Figure 1), whereas 6 (7%) patients had isolates with identical patterns in three different clusters (2 patients in each cluster) (Figure 2). Two patients who

Table 1. Demographic and Clinical Characteristics by Restriction Fragment Length Polymorphism Patterns

Characteristic	*Clustered (n = 6)*	*Nonclustered (n = 79)*	*Total (n = 85)*
Mean age	59	54	54
Male sex (%)	6 (100)	57 (72)	63 (74)
Residence			
Taipei County	5	38	43 (50)
Taipei City	0	21	21 (25)
Others	1	17	18 (21)
Unknown	0	3	3 (4)
Underlying diseases (%)*			
Diabetes mellitus	0	19	19 (22)
COPD	0	3	3 (4)
CLD	2	2	4 (5)
CRF	0	3	3 (4)
Pneumoconiosis	0	3	3 (4)
Rheumatoid arthritis	0	2	2 (2)
Corticosteroid use	0	2	2 (2)
Miscellaneous†	1	5	6 (7)
None	3	47	50 (59)

*Some patients had more than one underlying diseases; †Includes one case each of cerebrovascular disease, Cushing syndrome, hepatoma, Down syndrome, lung cancer, and myelodysplastia.
COPD = chronic obstructive pulmonary disease; CLD = chronic alcoholic liver disease; CRF = chronic renal failure.

had the same cluster isolates, lived in the same small village. The patients from the other two clusters had no demographic relationship or evidence of close contact. There were other strains that had similar patterns with only one or two band differences (see Figure 2). However, the patients with these strains also showed no obvious demographic or epidemiologic relationships.

Among patients with isolates that belonged to cluster RFLP patterns, two had chronic alcoholic liver disease, one had chronic renal failure, and three had no known underlying disease. In the nonclustered group, 19 patients (24%) had diabetes mellitus, and 44 patients (56%) had no known underlying disease (see Table 1). All of the patients with noncluster isolates and none of those with cluster isolates lived in Taipei City. The copy numbers of IS*6110* ranged from 3 to 20 (mean, 12) (Figure 3).

DISCUSSION

The Taiwan Provincial Tuberculosis Control Bureau has conducted national tuberculosis prevalence surveys every 5 years since 1957.[1] The data from the most recent survey show that the sex ratio (2.9:1, male to female) and age distribution of the reported cases were similar to those of patients identified in this study. The cases evaluated in this study represented nearly 5% of all cases of tuberculosis reported in the Taipei City and County region during the same period and 25% of the total number of tuberculosis cases diagnosed at Mackay Hospital. Hence,

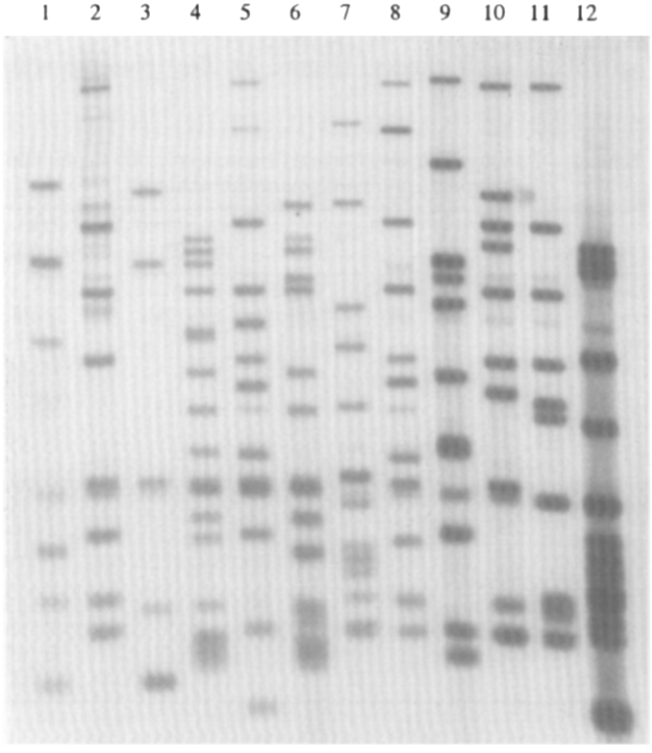

Figure 1. RFLP patterns of clinical isolates (lane 1–11) and H37Rv (lane 12) showing diversity of all isolates.

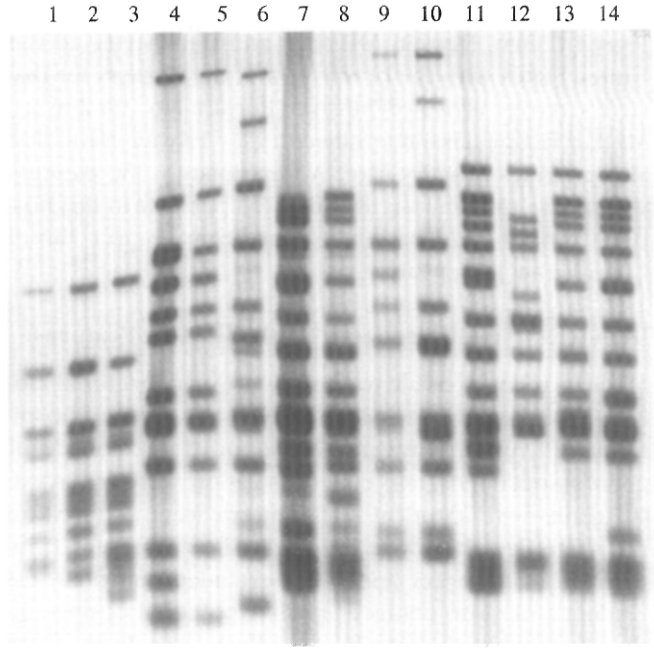

Figure 2. DNA fingerprinting with two clusters of identical patterns (lane 7 and 8 and lane 9 and 10, respectively). There were strains with similar patterns that were epidemiologically unrelated (lanes 1–3, 4–6, and 11–14, respectively).

the observations made in this study are representative of the tuberculosis situation in this region as a whole.

The repetitive DNA element IS*6110* is found in the genome of *M. tuberculosis* strains in a variable number of copies and at different locations.[3-6,9] Several other studies of *M. tuberculosis* isolates from Asian countries have reported that some strains possess none or only one copy of this element.[17-19] In the present study, most strains had between 5 and 20 copies (see Figure 3). The differences in copy numbers may represent differences in the local geographic distribution of related strains.

Although the prevalence of tuberculosis remains relatively high in Taiwan, the incidence of culture-confirmed cases has been declining steadily since the 1950s. The finding, in the present study, that most cases of tuberculosis (93%) were caused by strains with unique RFLP patterns

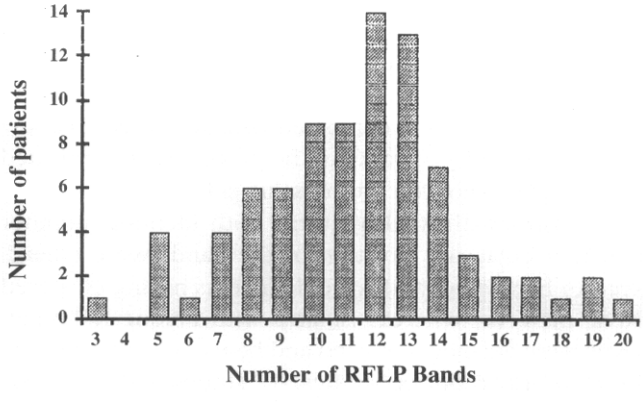

Figure 3. Distribution of IS*6110* copy number of the *M. tuberculosis* isolates (70% had copy numbers between 8 and 14).

suggests that reactivations rather than new exogenous transmissions were the major determinant of the cases, and the high proportion of patients with underlying medical problems provides further support for this conclusion.

In contrast to the findings in San Francisco and New York, the proportion of tuberculosis cases with *M. tuberculosis* isolates having identical or cluster RFLP patterns in Taiwan was small (7%). In recent studies from American cities, such strains represented about 40% of the clinical isolates.[14-16] These differences may be influenced by the prevalence of HIV infection in the different communities. On the entire island of Taiwan, only 540 cases of HIV infection were reported through 1993.[20,21]

Human immunodeficiency virus infection appears to amplify the incidence of primary tuberculosis by increasing the likelihood of disease manifestation after recent infection with *M. tuberculosis*. Therefore, HIV infection enlarges the reservoir of sources of transmission of *M. tuberculosis*. It is reasonable to expect that the potential impact of HIV on tuberculosis incidence will be greatest in communities with a relatively high prevalence of tuberculosis.

A sensitive way to detect changes in the transmission patterns of tuberculosis may be to monitor the proportion of tuberculosis cases that are attributable to strains with cluster RFLP types. The observation in American cities that the increase in the incidence of tuberculosis is associated with a large percentage of the strains having cluster RFLP types suggests that a substantial proportion of the increase may be attributable to recent exogenous transmissions.[14-16] Therefore, a rise in the percentage of strains with cluster RFLP types might serve as an early warning of an increase in the overall incidence of tuberculosis in a community. With the recent indications that the incidence of HIV infection is rising in Taiwan, this type of monitoring scheme might be an important component of tuberculosis control strategies.

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